MICROBIAL COMMUNITY STRUCTURE IN A UNLIMED AND LIMED MINE CONTAMINATED SOIL (Pb, Cu, As) WITH DIFERENT ORGANIC AND INORGANIC TREATMENTS

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1 INTRODUCTION

Mine contaminated soils are very unfavourable environments with limiting factors, in particular residual high levels of heavy metals, soil acidity, lack of organic matter and poor substrate structure. Toxic effects of HM on soil microorganisms have been extensively studied (Frostegård *et al.*, 1993; Bååth *et al.,* 1998) and the measurements of community structure indicated that the HM had an effect resulting in a change in community composition (Ellis *et al.,* 2003; Rajapaksha *et al,* 2004). Nowadays molecular biology techniques, such as the analysis of phospholipid fatty acid (PLFA) patterns, make it possible to study the microbial community structure of soil microorganisms. The PLFA technique has been used to elucidate different strategies employed by microorganism to adapt to changed environmental conditions under wide ranges of soil types, management practices, climatic origins and different perturbations (Zelles, 1999). By phospholipid fatty acid analysis it is possible to examine broad scale patterns in microbial community structure (Bååth *et al.* 2005) and generally, after the application of multivariate statistical analyses, whole community fatty acids profiles indicate which communities are similar or different. Determination both microbial community composition and biomass size by this direct method gives results that very closely represent the *in situ* soil conditions and is currently used for soil monitoring purposes (Nielsen and Winding, 2002).

This study aimed to evaluate the effect of different remediation technologies in a mine contaminated soil including several organic and inorganic treatments combined with liming by the soil microbial community structure analysis.

2 MATERIALS AND METHODS

The study was performed with a sandy loam soil with 3.7 pH value and contaminated with As, Pb and Cu (Table 1) from São Domingos mine (South of Portugal). Three replicated (48 pots) with 2 kg of sieved soil were used. Liming was performed in a half of the samples to rise pH and to compare with un-limed ones. Both of them were combined with the following treatments: inorganic fertilizer (I, 0.1 g N, 0.1 g P, 0.21 g K and 0.03 g Mg kg^{-1} soil); polyacrylate polymers and inorganic fertilizer (P, 0.1% of polyacrylate polymer with 210 mg K^+ g^{-1} as counter and 0.1% of a polyacrylate polymer with 98 mg $NH4^+$ g^{-1} polymer, 0.1 g P and 0.03 g Mg kg^{-1} soil); inorganic fertilizer plus an organic amendment with municipal solid waste compost (MSWC) (O, 0.1 g N, 0.1 g P, 0.21 g K, 0.03 g Mg kg^{-1} and 30 g MSWC kg^{-1} soil); inorganic fertilizer, polymer and organic amendment (PO, 0.2% of polyacrylate polymer as before, 0.1 g P and 0.03 g Mg and 30 g MSWC kg^{-1} soil). All the pots were sown with perennial ryegrass and irrigated periodically with de-ionized water. The measurements of PLFA pattern were made on soil samples collected at two different times (1 and 4 months) after plant cultivation and application of the treatments.

The microbial community structure was determined by phospholipid fatty acid (PLFA) analysis (Frostegard *et al*., 1993). Fatty acids (PLFA) are designated in terms of the total number of carbon atoms: number of double bounds, followed by the position of the double bound from the methyl end of the molecule. Cis and trans configurations are indicated by c and t, respectively. The prefixes a and i indicate anteiso- and iso-branching; br indicates unknown methyl branching position, 10Me indicates a methyl group on the tenth carbon atom from the

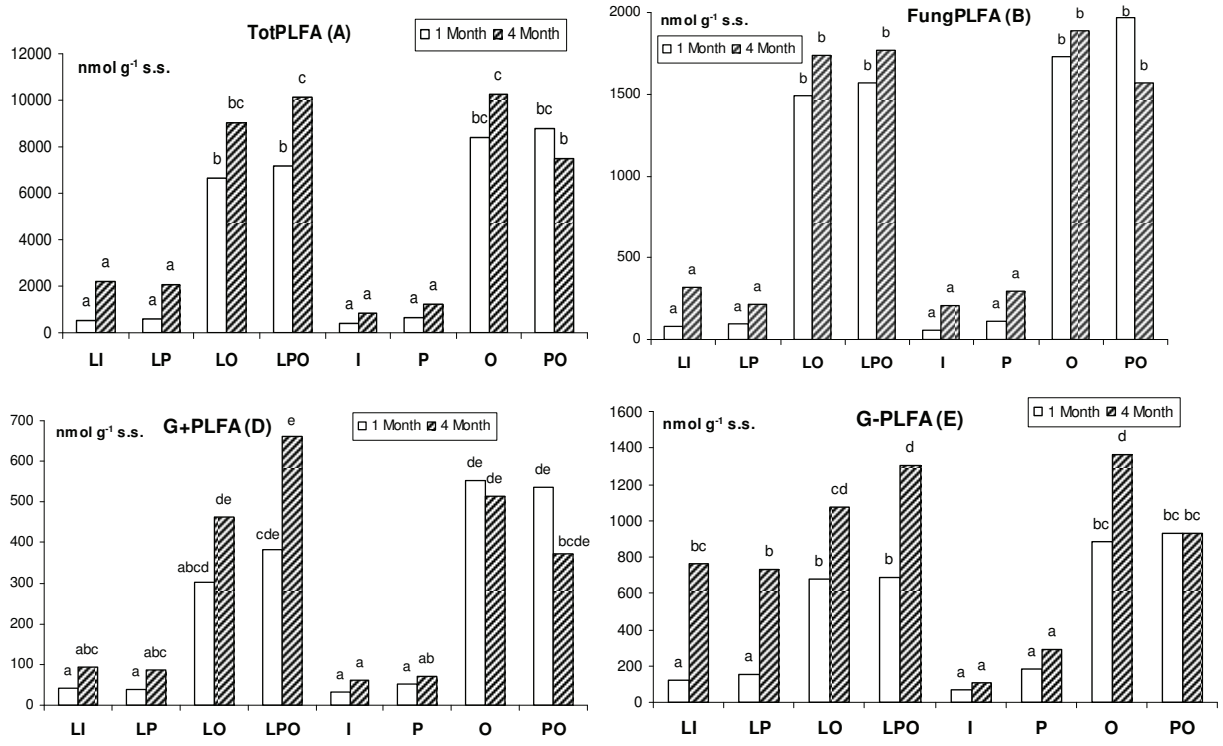
carboxyl end of the molecule; and cy refers to cyclopropane fatty acids. The total microbial biomass (TotPLFAs) was estimated as the sum of all the extracted PLFAs. The sum of the PLFAs considered to be predominantly of bacterial origin was used as an index of the bacterial biomass (BactPLFAs), the quantity of the PLFA 18:2ω6,9 was used as an indicator of the fungal biomass (FungPLFA), the PLFAs i14:0, a15:0, i16:0 and 10Me18:0 as indicators of gram-positive (G^+) bacteria, and the PLFAs cy17:0, cy19:0, 16:1ω7c and 18:1ω7 as indicators of gram-negative (G^-) bacteria (Díaz-Raviña *et al.,* 2006). Concentration of all the individual PLFAs data, expressed as mole percentage and logarithmically transformed, was subjected to principal component analysis (PCA) to elucidate the main differences in the PLFA patterns. To compare treatments, data were tested by ANOVA and Tukey´s minimum significant difference test was used to differentiate the means.

TABLE 1 **Some physico-chemical characteristics of initial soil samples**

	Texture	pH H_2O	MO (%)	Cu mg kg^{-1}	Pb mg kg^{-1}	As mg kg^{-1}
Initial soil	Sandy loam	3.7	0.6	288	33500	8250

3 RESULTS AND DISCUSSION

Fig. 1 shows that in both unlimed and limed soils, independently of sampling time considered, the total microbial biomass and the biomass of specific groups were significantly ($p < 0.05$) higher in the treatments with MSWC than in the other treatments. Furthermore, no differences were observed between limed treated samples and the corresponding unlimed controls**.**



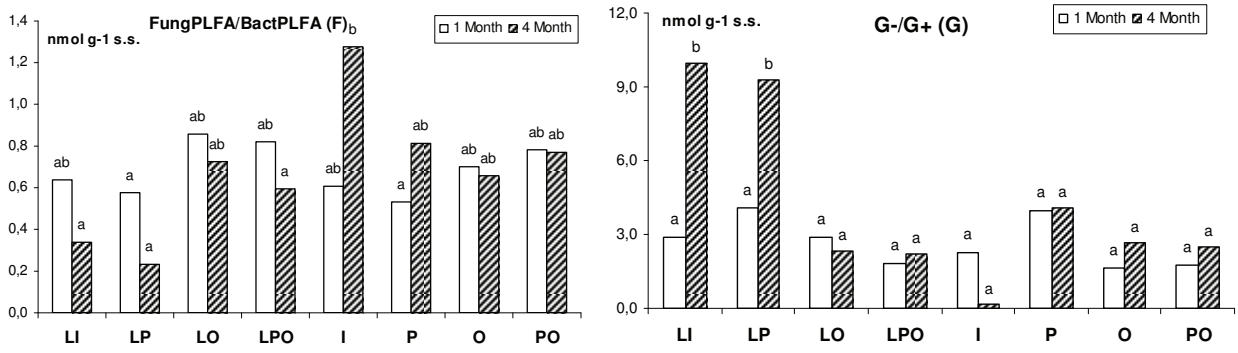


FIGURE 1 **Phospolipid fatty acid concentrations (mean values of three pot replicates) of mine soil with different reclamation treatments.** A, total PLFA (TotPLFA); B, fungal PLFA (FungPLFA); C, bacteria PLFA (bactPLFA); D, gram positive bacteria PLFA (G+ bactPLFA); E, gram negative bacteria PLFA (G- bactPLFA); F, fungal PLFA to bacteria PLFA ratio (FungPLFA/BactPLFA) and G, gram-negative to gram-positive BactPLFA ratio (G^-/G^+). Treatments: I, inorganic fertilizer; P, polymer; O, MSCW; PO; polymer and MSCW; LI, liming plus inorganic fertilizer; LP, liming plus polymer; LO, liming plus MSCW; LPO; liming plus polymer and MSCW. Different letters indicate significant differences at the P<0.05 level.

The PCA obtained (Fig. 2) showed that the first principal component (PC1) explained 43% of the total variance, thus, MSWC organic amendment had a more marked effect than inorganic and/or polymer, increasing the branched saturated PLFAs i16:0; a15:0; 16:0; 15:0; 20:0; 17:0; 10Me16:0 and monounsaturated PLFAs 16:1ω9, 16:1ω5, 19:1

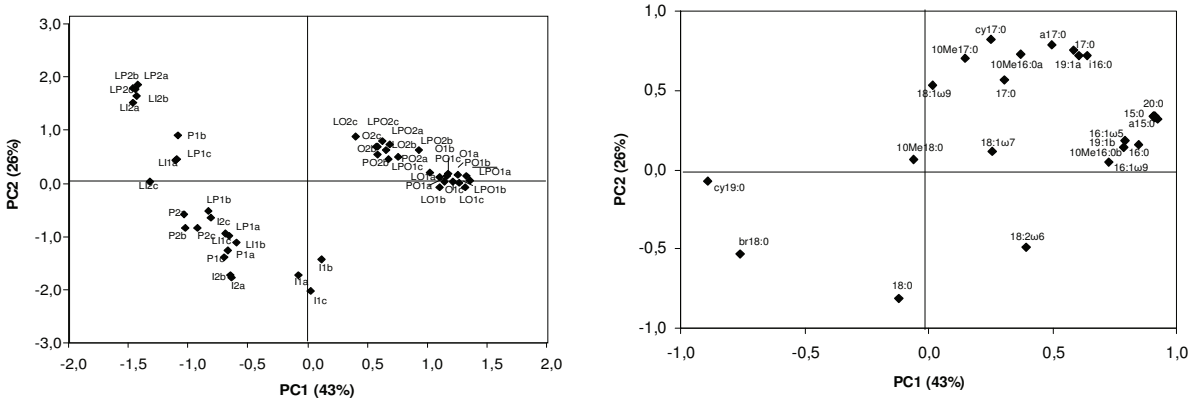


FIGURE 2 **Score and loading plots from PCA performed on the PLFAs of the mine soil. Treatment: I, inorganic fertilizer; P, polymer; O, MSWC; PO, polymer and MSWC; LI, liming plus inorganic fertilizer; LP, liming plus polymer; LO, liming plus MSWC; LPO, liming plus polymer and MSWC.**

4 CONCLUSIONS

The results clearly indicated that PLFA data allow us discriminate the different organic and inorganic treatments, the effect of MSWC organic treatments being more marked than those of inorganic treatments y/or polymer. Moreover, liming to increase pH showed no differences with the corresponding un-limed controls on basis of microbial community structure.

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